

## **Influencing upon Mammalian Radioresistance with Biologically Active Drug Respistim Plus**

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### **ABSTRACT**

*Radiobiology is a medical knowledge, which studies ionizing radiation and its influence over the human body. Military radiobiology investigates care to soldiers and civil population after radiation exposure. Acute radiation syndrome and its treatment are the main topics of military radiobiology research. These are the three main trends in Bulgarian military radiobiology:*

- *Keeping under control the first reaction after acute radiation exposure;*
- *Searching and investigating de novo synthesized chemical materials;*
- *Stem cells investigation and its clinical appliances;*
- *Stem cells investigation and its clinical appliances;*
- *Investigation of stem cells and especially its clinical application.*

*Over the past years different biological substances are applied with success. Protection of the hematopoietic and immune systems is a critical area for restoring organism after irradiation. Some radioprotective characteristics of biologically substances, vitamins and essential elements are established.*

*Aim of the study: To determine radioprotective effect of Bulgarian drug Respistim plus – a mixture of lyophilized bacterial strains in combination with vitamins and essential elements on the proliferation and migration of hematopoietic stem cells, levels of white blood cells and increased 30 days survival after irradiation.*

*Materials and methods: We investigate groups of experimental animals C3H mice, which are exposed to radiation in different doses. Respistim plus is administered to some of those experimental animals per os 15 days before irradiation. Mice are irradiated in <sup>137</sup>Cs gamma radiation source at a dose rate of 126Gy/h. We control the following indexes: survival rate, blood indexes, weighs of some body organs, bone marrow cells vitality.*

*The effect of Respistim plus is evaluated with probit analysis of survival rate and Kaplan-Meier survival analysis; bone marrow cells vitality test with trypan blue stain, changes of blood indexes. Statistical data treatment is done by methods of variational analysis and we consider as statistically reliable the results with  $p < 0,05$ .*

*Conclusions:*

*Administration of Respistim plus increases LD50/30 survival rate to 8,4Gy, in comparison with control group - 7,5Gy.*

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*Administration of Respistim plus saves bone marrow cellularity according to the bone marrow vitality test.*

*Administration of Respistim plus stimulates proliferation of hematopoietic stem cells and has positive impact in preserving weight of some body organs.*

Radiobiology is a medical science dealing with the impact of radiation on living matter. Ionizing radiation possesses some characteristics especially occurring in their interaction with living organisms: no subjective sensations during irradiation [1, 2, 3]; had a latent period after irradiation [11]; post irradiation recovery is incomplete. Pathogenetic mechanism of radiation damage is confined to a chain of successive processes, and changes in the structure and function of DNA, proteins, cell death, mutations [1, 10]. At the heart of these processes are generated free radicals. Oxidative damage is the basis of later morphological changes in tissues and organs. Limiting oxidative damage would assist in the rapid recovery of the irradiated organism. Recently the clinicians and researchers show an interest in the mechanisms of protective action of antioxidant substances and the possibility of their use for the prevention and treatment to so-called free radical diseases [6, 7].

Radioprotective agents are compounds that are administered before exposure to ionizing radiation to reduce its damaging effects, including radiation-induced lethality [2, 5]. Naturally occurring dietary components offer opportunities for development as effective chemopreventive and radioprotective agents because of their low toxicity [8, 9, 12, 13]. Respistim plus - an original Bulgarian drug is a mixture of lyophilized bacterial strains in combination with vitamins and essential elements. It has strong antioxidant activity and many beneficial health effects.

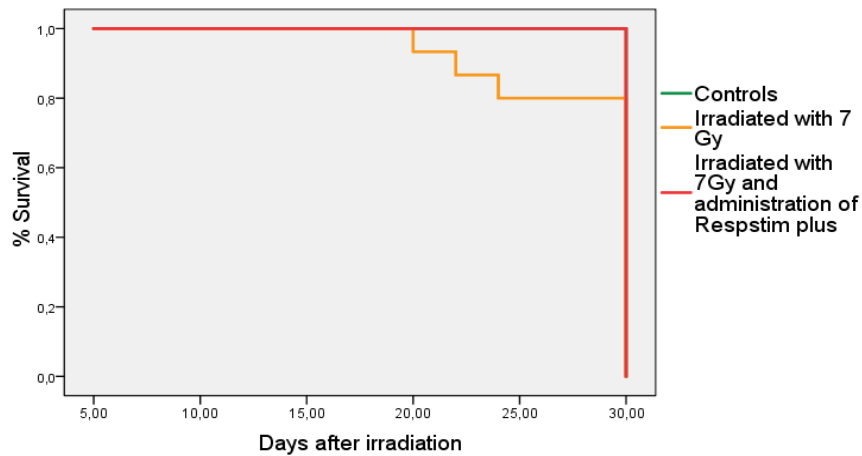
THE AIM OF THE STUDY is to determine radioprotective effect of Bulgarian drug **Respistim plus** – a mixture of lyophilized bacterial strains in combination with vitamins and essential elements on the proliferation and migration of hematopoietic stem cells, increased levels of white blood cells and increased 30 days survival after irradiation.

#### MATERIALS AND METHODS

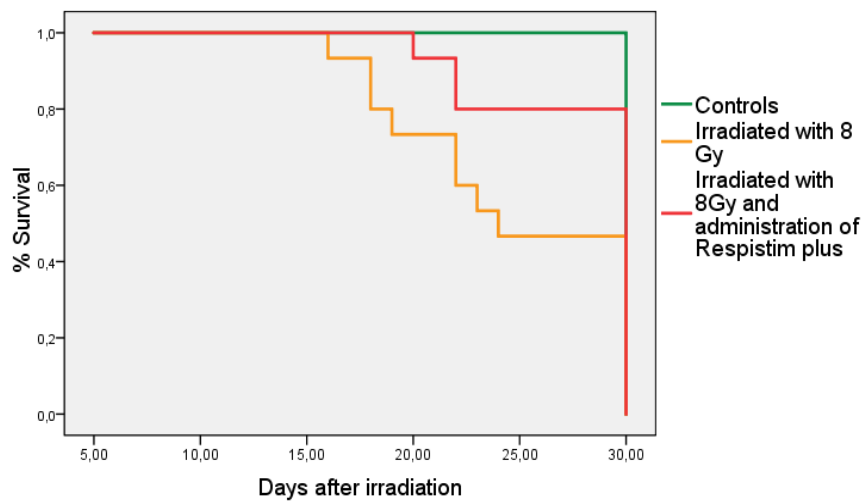
Male C<sub>3</sub>H mice weighing 24-28g were used in these studies. Mice were housed in a facility accredited by Ethical Committee of Laboratory Animal Care. Animal rooms were maintained at 21<sup>0</sup> ± 5<sup>0</sup>C on a 12-hr light/dark cycle. We investigate groups of experimental animals C<sub>3</sub>H mice, which were exposed to radiation in different doses (7, 8, 9, 10Gy). Respistim plus is administered to some of those experimental animals *per os* 15 consecutive days before irradiation, with doses 100mg/kg. Mice were irradiated in <sup>137</sup>Cs gamma radiation source at a dose rate of 126Gy/h. Bone marrow cells were isolated from femurs with 0.5ml 0,9%NaCl, stained and counted. Blood indexes were calculated automatically using hemocytometer PCE-170 (Erma INC, Japan). Survival analysis were made with probit analysis and Kaplan-Meier method. Statistical data treatment was done by methods of variational analysis and we consider as statistically reliable the results with p<0,05. Statistical evaluation was made with SPSS for Windows.

#### RESULTS

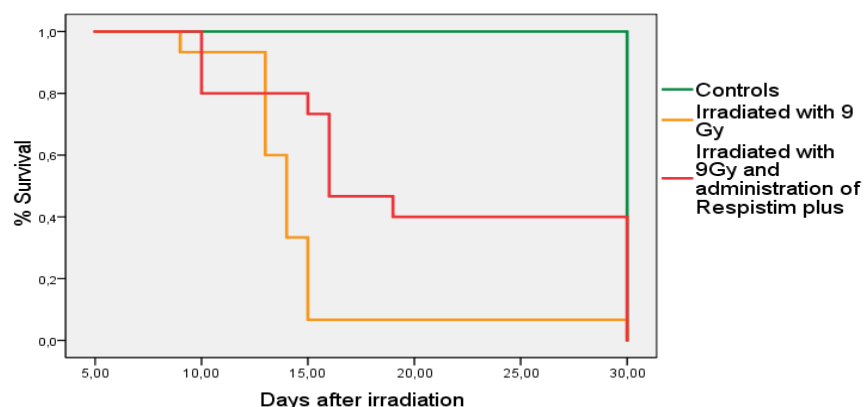
Our results shows that administration of Respistim plus for 15 consecutive days before irradiation, enhance 30-day survival for groups of mice that received drug in dose of 100 mg/kg (Fig.1, 2, 3 and 4).



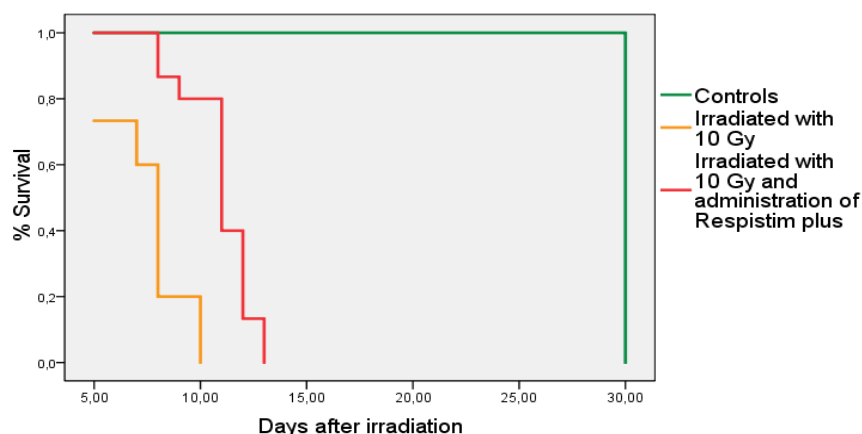
**Fig.1: Survival curves for mice with administration of Respistim plus (red), irradiated group (orange) and controls (green). Mice are irradiated in  $^{137}\text{Cs}$  gamma radiation source at a dose rate of 126Gy/h. Survival was followed in the 5, 10, 15, 20, 25 and 30th day post irradiation.**



**Fig.2: Survival curves for mice with administration of Respistim plus (red), irradiated group (orange) and controls (green). Mice are irradiated in  $^{137}\text{Cs}$  gamma radiation source at a dose rate of 126Gy/h. Survival was followed in the 5, 10, 15, 20, 25 and 30th day post irradiation.**



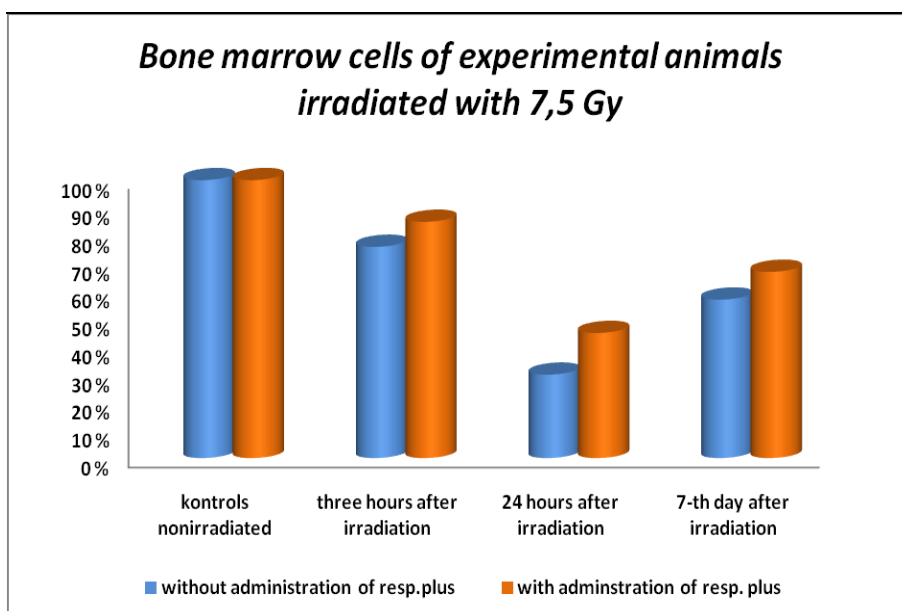
**Fig.3: Survival curves for mice with administration of Respistim plus (red), irradiated group (orange) and controls (green). Mice are irradiated in  $^{137}\text{Cs}$  gamma radiation source at a dose rate of 126Gy/h. Survival was followed in the 5, 10, 15, 20, 25 and 30th day post irradiation.**



**Fig.4: Survival curves for mice with administration of Respistim plus (red), irradiated group (orange) and controls (green). Mice are irradiated in  $^{137}\text{Cs}$  gamma radiation source at a dose rate of 126Gy/h. Survival was followed in the 5, 10, 15, 20, 25 and 30th day post irradiation.**

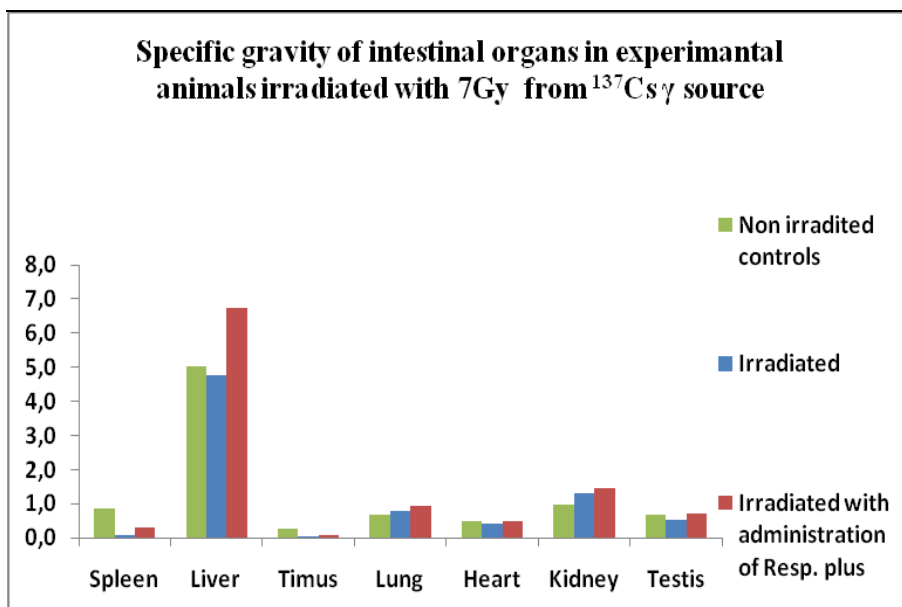
With method of probit analysis we calculated  $\text{LD}_{50/30}$ , for Respistim plus-treated group on 8,5 Gy, controls are with 7,5 Gy.

Bone marrow cells are very sensitive for irradiation influence. Bone marrow cells lethality enhanced in the first 24-hr. after radiation exposure. For investigating influence of Respistim plus over bone marrow cells, we used trypan blue stain evaluation method (Fig.5).



**Fig.5: Bone marrow cells vitality in presenting of mice irradiated whit a single dose 7,5 Gy. Mice were evaluated immediately, 24 hours and on the 7th day after irradiation. Bone marrow cells were presented in percentage.**

Changes in relative weight of intestinal organs are a marker of cell loss after irradiation exposure. Our experimental data shown that this weight in spleen and thymus are decrease with high degree in comparisson with controls (irradiated and nonirradiated).



**Fig.6 Specific gravity of intestinal organs (spleen, liver, thymus, lung, heart, kidney, testis) from experimental animals on the 5th day after irradiation with 7Gy. Specific gravity was presented in percentage.**

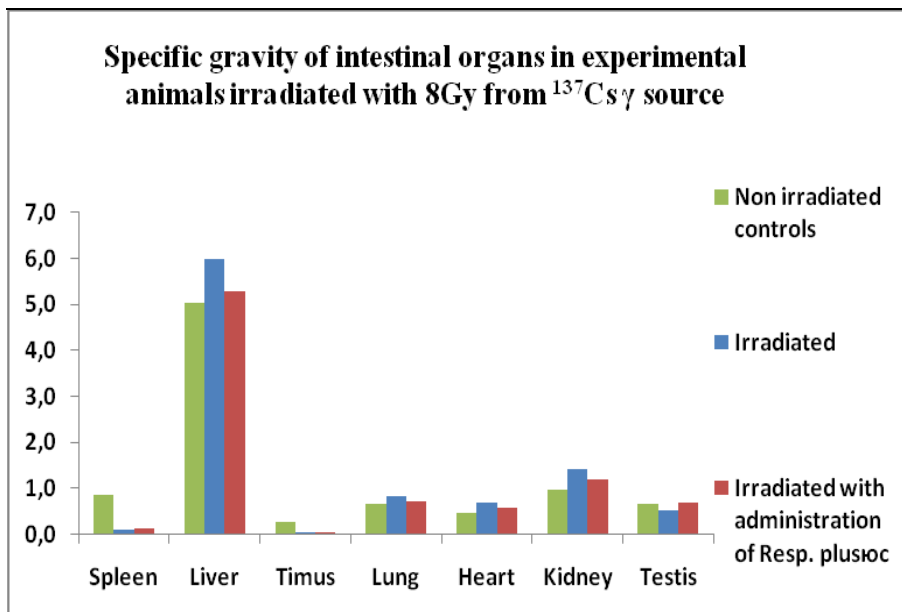


Fig. 7 Specific gravity of intestinal organs (spleen, liver, thymus, lung, heart, kidney, testis) from experimental animals on 5th day after irradiation with 8Gy. Specific gravity was presented in percentage.

Leucocytes number counted in peripheral blood is also a sensitive marker for severity of radiation exposure [4]. We examined a white blood cells count on different intervals after exposure (Fig.8).

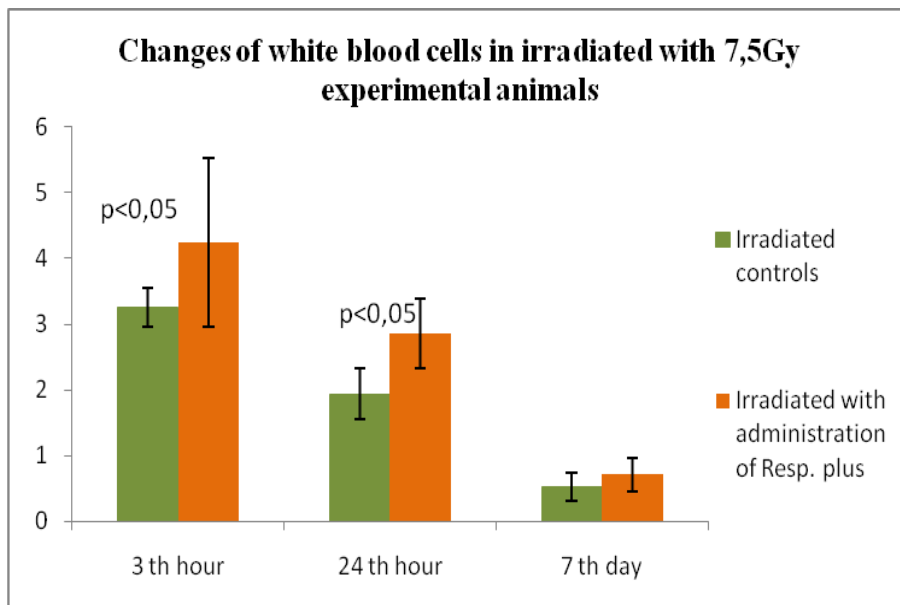


Fig.8 Changes in leucocyte absolute count in experimental animals irradiated with a single dose 7,5Gy from  $\gamma$ -irradiation source  $^{137}\text{Cs}$ .

On fig. 8 are presented the changes of white blood cells. The result shows that the applying of Respistim plus increase the level of cells on 3th hour post irradiation, on the 24 hour as well as on the 7<sup>th</sup> day.

## DISCUSSION

Survival is an integral indicator of effectiveness of a given substance in radiobiology investigation [14]. Earlier death (3-7 day) is an indicator of combined gastrointestinal and hematopoietic radiation injury among irradiated experimental animals. Cell destruction is an important link in the development of radiation sickness. This destruction connects with pathological changes of functional tissues: spleen, liver, bone marrow. Changes in the relative weights are clearly in the spleen and thymus. Spleen is important organ for the hematological system [14]. Changes after irradiation in the spleen are described, and include destruction of lymphoid follicles, gradually throughout the white pulp as degenerative changes occur almost immediately after exposure (30 minutes) in the germ centers, bleeding in the red pulp, followed by a phase of active phagocytosis. Due to the rapid and extensive destruction of cells, the spleen is reduced in size and weight. Our results show that in thymus changes in relative weight in the early time is in good connection with a significant cell loss too.

Bone marrow vitality cells (Fig.8) are indicative of regeneration capabilities of haematopoietic stem cells. There were differences among group irradiated and treated with **Respistim plus** and control irradiated group (Fig. 8).

The results of this study demonstrate that, a dose of 100 mg/kg **Respistim plus** mitigate the lethal effects of radiation exposure. It possesses a number of biological properties that may contribute to its radioprotective efficacy. These effects may be including eventually antioxidant or/and radical scavenging properties of the drug as well as immunostimulatory activity.

In conclusion, our research demonstrates that **Respistim plus** is an effective nontoxic radiation protective agents against radiation-induced lethality in mice.

Administration of **Respistim plus** saves bone marrow cellularity according to the bone marrow vitality test.

Survival rate of the experimental group ( $LD_{50/30}$ ) with administration of **Respistim plus** increases to 8,4Gy, in comparison with control group - 7,5 Gy.

Three and twenty-four hours after 7,5Gy radiation we evaluate statistically reliable ( $p<0,05$ ) increasing of leucocytes in peripheral blood. This increased number of peripheral leukocytes can be associated with increased numbers of stem cells in bone marrow.

Ionizing radiation has been shown as an pathogenic mechanism of disturbances, including enhance the production of reactive oxygen species in a variety of cells, tissues and organs and develop of oxidative stress. Our results show very good prevention of **Respistim plus** on the disturbances causes from this experimental model of irradiation syndrome. The mechanism(s) of this prevention is the subject of our future research. We have some hypothesis about it:

- Activation of stem cells proliferation and migration;
- Decreased oxidative injuries from ROS in liver, spleen, blood plasma;
- Increased levels of antioxidant enzyme SOD1 and metalotioneines;

The results obtained with the described experiments suggest that the observed effect of **Respistim plus** may be is connected with its direct effect upon the free radical processes in organism.



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